ISSN: 0974-5343
IJMST (2011), 4(1):1-5

Utility of Gamma Glutamyltransferase and AST/ALT (De Ritis) ratio in Alcoholic liver diseases

Dr.Vidya.S.Patil[1], Dr. P.B.Desai[2], Dr. M.V.Kodliwadmath[3], Dr.Indumati.V[4]

1Department of Biochemistry, SDM College of Medical Sciences and Hospital, Dharwad, Karnataka, INDIA
2Department of Biochemistry, J.N Medical College, Belgaum, Karnataka, INDIA
3Department of Biochemistry, Navodaya Medical College, Raichur, Karnataka, INDIA
4Department of Biochemistry, Vijayanagara Institute of Medical Sciences, Bellary, Karnataka, INDIA
vidyashankar02@yahoo.co.in

ABSTRACT:
Aim: To assess the value of enzymes Gamma glutamyltransferase (GGT), Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) as diagnostic indicators of alcoholic liver diseases. Material and Methods: Our study group comprised of 25 normal healthy controls, 50 patients with advanced alcoholic liver disease (ALD), 15 patients with acute viral hepatitis (AVH) and 10 patients with non alcoholic cirrhosis (NALD). We analysed GGT, AST, ALT, Total bilirubin, Total protein, Albumin and Prothrombin time. AST/ALT ratio and discriminant function were calculated. Results: GGT values were significantly high (6-8 times upper limit of mean of normal controls) among ALD patients in comparison with all other groups. Mean AST/ALT ratio among ALD patients was >2. 88% patients with ALD had AST/ALT ratio of ≥1.5. The ratio was<2 among all the other diseased groups with a value of 1.15 among normal healthy controls. Discriminant function score of ≥32 was found in 9 among 50 ALD patients. Conclusion: GGT and AST/ALT ratio of ≥1.5 together are good indicators of alcohol as the cause of liver disease. AST/ALT ratio >2 indicates advanced liver disease in alcoholics. Bilirubin and prothrombin time can be used to know the severity of liver disease as a part of discriminant function. A discriminant function of ≥32 have poor prognosis and also helps to select patients for steroid therapy.

Key words: Alcoholic liver disease, AST/ALT ratio, GGT, Discriminant function, Prothrombin time.

INTRODUCTION

Alcoholism and hence alcohol related problems are a major problem in western world and is of growing concern in developing countries. The prevalence of alcohol intake is reported as 10.4% (5-20%) in men in India [1]. Alcoholic liver disease (ALD) is the predominant form of chronic liver disease as reported by Narawane NM et al. Liver involvement appears earlier with lower consumption of illicit liquor in Indians than in west [2]. An alarming trend is the increase in alcohol abuse even among women and children. Women have been found to be twice as sensitive to alcohol – mediated hepatotoxicity and may develop more severe ALD at lower doses and with shorter duration of alcohol consumption than men [3]. The amount of alcohol ingested is the important risk factor for the development of ALD. The risk of developing cirrhosis increases with ingestion of >60-80 g/day for >10 years in men and >20 g/day in women [4].

ALD is increasingly seen in countries such as Japan and India which traditionally had a low prevalence of the disease[5]. The presence and extent of protein calorie malnutrition have an important role in determining the outcome of patients with ALD. Mortality increases in direct proportion to the extent of malnutrition, approaching 80% in patients with severe malnutrition [6]. In addition, genetic factors predispose to both alcoholism and ALD. Polymorphisms of genes involved in the metabolism of alcohol and in those that regulate endotoxin-mediated release of cytokines have been associated with ALD [7]. Genetic polymorphisms of gamma glutamyltransferase (GGT) may link with the induction of GGT by alcohol and consequently to the development of alcoholic liver and pancreatic diseases [8]. A public health strategy for ALD would identify patients at high risk of developing cirrhosis and its complications and would refer such patients for abstinence – promoting therapy.

Laboratory tests need to be a part of the diagnostic process for confirming the diagnosis of alcohol abuse, follow up of patients undergoing treatment and in monitoring abstinence. The conventional marker gamma glutamyltransferase (GGT) continues to remain the test combining greatest convenience and sensitivity. Its diagnostic accuracy can be enhanced by combination with other traditional markers such as aminotransferases (AST, ALT) and certain parameters which indicate the severity and prognosis like Total proteins, albumin, bilirubin and prothrombin time [9]. No single laboratory marker definitively establishes alcohol to be the cause of liver disease. Combining various biochemical parameters is more sensitive and specific in ALD than a single biochemical abnormality. Hence, in this study, an attempt is made to correlate various biochemical parameters with ALD pattern.

MATERIAL AND METHODS

The present study was carried out at Department of Biochemistry, J.N Medical College, Belgaum. Our study group comprised of a total of 100 participants which included Group 1 with 25 normal healthy persons as control group, Group 2 with 50 patients having advanced alcoholic liver disease and Group 3 with 25 patients of non-alcoholic liver disease (3a - 15 among these were having acute viral hepatitis and 3b -

10 were with non-alcoholic cirrhosis of liver). All were between the age group of 30-60 years.

Inclusion criteria: Patients with a history of chronic alcohol consumption of not less than an average of 40g daily for atleast 10 years with the presenting features like tender hepatomegaly, jaundice, ascitis or gastrointestinal bleeding were included in the study group 2 (advanced alcoholic liver disease). All subjects were evaluated and selected by detailed medical history and physical examination. Each subject gave informed consent and the study was approved by Institutional ethical and research committee.

Methods:
5 ml of venous blood sample was collected with aseptic precautions in both plain and citrated bulbs. Serum was separated by centrifuging at 3000rpm for 15 min and was stored at 4^0C for estimation of enzymes, bilirubin, protein and albumin which was preferably done within 2 hrs. Plasma obtained from centrifuging citrated blood was used to perform prothrombin time which was done immediately.

Following parameters were analysed:

1. Gamma glutamyltransferase (GGT) – Szasz method[10]
2. Aspartate and alanine aminotransferases (AST & ALT) – Reitman and Frankel method[11]
3. Serum Bilirubin – Malloy and Evelyn method[12]
4. Serum total protein – Biuret method[13]
5. Serum albumin – Dye binding method[14]
6. Prothrombin time – Quicks method [15]

Following calculations were made –

1. AST/ALT ratio (De Ritis ratio)
2. Maddrey Discriminant Function (MDF) = 4.6(Patient's PT-Control PT)+Total Bilirubin (mg/dl) [16]

Statistical analysis of all the parameters was done by student't' test and the significance values were obtained.

RESULTS
All the observations made in the study are shown in table 1. There was a significant increase in GGT, AST, ALT, AST/ALT ratio, Total bilirubin among groups 2,3a and 3b in comparison with normal healthy control group 1($p<0.001$). Similarly there was significant decrease in total protein, albumin and A:G ratio in all the diseased groups in comparison with healthy controls($p<0.001$). Prothrombin time was significantly prolonged among groups 2,3a and 3b as compared with group 1($p<0.001$).

We also made comparison between ALD patients with NALD patients. We observed that GGT was significantly increased among ALD patients when compared with both groups of NALD (AVH and cirrhosis) patients ($p<0.02$, $p<0.001$ respectively).

The most striking finding was AST/ALT ratio which was >2 in ALD patients which was significantly more in comparison with AVH group ($p<0.001$), but was not so with non alcoholic cirrhosis group. Still the AST/ALT ratio in non alcoholic group was <2 even though it was not significantly lesser than ALD group ($p>0.2$). Another notable finding was AST levels which were very much higher compared to ALT elevations among ALD patients, due to which the AST/ALT ratio was >2 in this group. The AST/ALT ratio of ≥2 was observed among 26 subjects out of 50 ALD patients (52%) and when the cut off value of ≥1.5 was considered, then 44 patients out of 50 ALD subjects (88%) were included. The ratio of ≥1.5 was observed in 88% of ALD patients, 80% of non-alcoholic cirrhosis patients and in only 20% patients with AVH. Similarly, a ratio of ≥2 was observed in 52% of ALD patients, 40% of non-alcoholic cirrhosis patients and in none of the patients with AVH. (Table 2). All the normal healthy controls had AST/ALT ratio in the range of 0.9 to1.4. There was no much significant differences among the groups with respect to other parameters except total bilirubin which was significantly higher among AVH patients compared to the other two groups of ALD and non alcoholic cirrhosis ($p<0.001$). Maddrey discriminant function was calculated and the value of ≥32 was found in 9 among 50 patients with ALD.

DISCUSSION
Liver disease due to excessive alcohol intake is a common medical problem encountered associated with tremendous mortality and morbidity. Several studies have been conducted to assess the usefulness of various laboratory tests in the diagnosis and prognosis of ALD. Our study estimated conventional marker GGT, traditional markers such as aminotransferases (AST and ALT) and also certain prognostic markers such as bilirubin, total protein, albumin and prothrombin time.

Serum GGT activity is increased in hepatobiliary disorders and with fairly heavy consumption of alcohol. In our study, GGT was significantly elevated in patients with ALD when compared to normal controls, patients with AVH and also non alcoholic cirrhosis. GGT exists to a large extent in smooth endoplasmic reticulum and is therefore subject to hepatic microsomal induction by drugs and alcohol. Because of the effects of alcohol on GGT activity, GGT assays are considered sensitive indicators of alcoholism [17].

IJMST

ISSN: 0974-5343
IJMST (2011), 4(1):1-5

Table 1 – Comparison of all the parameters between groups 1, 2, 3a and 3b (All values expressed as Mean±Standard deviation)

Parameters	Group 1 Controls n = 25	Group 2* ALD n = 50	Group 3a# NALD - AVH n = 15	Group 3b$ NALD cirrhosis n = 10	P value
GGT IU/L	19.85±5.03	120.43±46.44	87.86±33.24	33.57±13.55	§ - <0.02 † - <0.001
AST U/ml	28.00±6.32	85.36±36.58	81.53±24.52	79.10±28.9	§ - >0.6 † - >0.6
ALT U/ml	24.88±5.35	40.9±10.8	65.8±17.11	43.6±10.78	§ - <0.001 † - >0.4
AST/ALT Ratio	1.15±0.26	2.08±0.63	1.24±0.24	1.81±0.46	§ - <0.001 † - >0.2
T.Bilirubin mg/dl	0.77±0.16	3.11±2.41	5.85±2.68	2.79±1.62	§ - <0.001 † - >0.6
T.Protein g/dl	6.82±0.34	5.51±0.64	5.64±0.37	5.27±0.36	§ - >0.4 † - >0.2
S.Albumin g/dl	3.45±0.17	2.26±0.39	2.37±0.31	2.13±0.16	§ - >0.3 † - >0.3
A:G ratio	1.03±0.09	0.71±0.17	0.73±0.12	0.67±0.09	§ - >0.7 † - >0.4
P.T Seconds	13.52±1.23	17.56±2.31	15.47±1.46	17.6±1.78	§ - <0.005 † - >0.9

$p<0.05$ – statistically significant, $p>0.05$ – not significant

*$p<0.001$ for all parameters between group 1 and 2

#$p<0.001$ for all parameters except AST/ALT ratio for which $p>0.3$ between group 1 and 3a

$ $p<0.001$ for all parameters between group 1 and 3b

§ - comparison between group 2 and 3a

† - comparison between group 2 and 3b

Table 2 – Comparison of AST/ALT ratio among different patient groups.

AST/ALT ratio	ALD (n=50)	AVH (n=15)	NALD (n=10)
≥2	26 (52%)	0 (0)	4 (40%)
≥1.5	44 (88%)	3 (20%)	8 (80%)
≥1	50 (100%)	12 (80%)	9 (90%)
≤1	0 (0)	3 (20%)	1 (10%)

The elevation was 6-8 times ULN in our study as shown by Jacobs WLW [18]. Baral N et al reported that a GGT level of ≥ 25 IU/L was significantly associated with ALD [19]. Pasqualetti et al (1995), in their study found that the patients with alcoholic liver diseases present significantly higher values of GGT in respect to patients with NALD. A significant decrease of about 50% in serum GGT levels was observed after alcohol withdrawal only in ALD patients and not in NALD patients. Hence they concluded that, even if GGT per se appear as weak indicator of alcoholism during chronic liver diseases, the early decrease in their values are good and specific markers of alcohol abuse, consequently of the alcoholic etiology of the disease [20]. Among chronic alcoholics, liver disease is assumed if GGT activity initially is 8-10 times normal and if the elevation persists after 6-8 weeks of abstinence from alcohol. On the other hand, if initial GGT levels are only 2-3 times normal and return to normal after abstinence, the patient is assumed to be free of liver disease. In the later case, increase of GGT is considered to be due to the inductive effect of ethanol on the synthesis of enzyme in liver [21].

In AVH, GGT is almost invariably raised with peak values averaging 4 times ULN, as was observed in our study. This and the increase in activity at the time of clinical recovery suggest that the enzyme elevation results from increased enzyme synthesis as an inflammatory response [22]. The diagnostic value of GGT in acute hepatitis is slight, while the aminotransferases give much better insight into the development of the disease [18].

Serum AST and ALT are often increased in patients who are alcoholics, although generally not to more than 2-4 times ULN [9]. Similar findings were observed in our study. Excessive alcohol consumption can lead to raised levels due to increased cell membrane permeability and cell necrosis. Serum levels depend markedly on the degree of liver damage and how recently alcohol has been consumed [9,23]. Our results show that the elevation in ALT is not as high as that of AST in ALD patients reflecting diminished hepatic activity of these enzymes, less enzyme being available to leak into the serum from damaged hepatocytes as shown by Maltoff DS et al [24] and

Cohen JA et al [25].The normal activity of AST and ALT found in some of our patients with ALD could be related to depletion of vitamin B1 and B6 as suggested by studies of Borghi E et aland Diehl et al [26, 27].

The ALT activity was found to be significantly higher in AVH patients when compared to ALD ($p<0.001$). But ALT levels did not exceed AST levels in AVH in our study. This is not in accordance with few studies which showed that ALT levels exceed far greater than AST in AVH [22, 25]. The elevated AST levels compared to ALT associated with AST/ALT ratio >1 in our study could suggest widespread cell necrosis of severity sufficient to liberate substantial mitochondrial AST and a poorer prognosis.

The utility of AST/ALT ratio (De Ritis Ratio) became widely recognised with the paper by Cohen and Kaplan in 1979[25]. In our study AST/ALT ratio was calculated which could help to differentiate between ALD and NALD patients. We found that AST/ALT ratio was >2 among ALD patients. The elevated AST/ALT ratio in ALD is due primarily to the selective and significant lowering of liver ALT by alcohol associated with increased AST levels from mitochondrial injury [24]. The ratio >1.5 strongly suggests, and a ratio >2 is almost indicative of alcohol induced liver damage [9]. AST/ALT ratio of >1 when ALT is < 300IU/L suggests ALD as seen in our study [21]. More than 80% of patients with ALD have an AST/ALT ratio of 2 or more [28].

In our study 52% patients with ALD had the ratio ≥2 and 88% had the ratio ≥1.5. An important observation was that the ratio of ≤1.0 was not found in any of the patients with ALD. Similarly, a study from Nyblom H et al showed that AST/ALT ratio increased significantly from patients with alcohol dependence(≤1.0 in 64% patients) to those with withdrawal symptoms and then to patients with cirrhosis and other complications (≥2 in 69% patients and ≤1.0 in 8% patients). Most patients with high alcohol consumption but without severe liver disease do not have an AST/ALT ratio above 1. High AST/ALT ratio suggests advanced alcoholic liver disease [29].

An increased AST/ALT ratio in patients with increased serum aminotransferase activity has also been associated with the development of cirrhosis in non-alcoholic steatohepatitis, even though still higher levels were reported in patients with ALD [30]. But there was an argument against the development of cirrhosis as the sole determinant for the AST/ALT ratio. Nyblom H et al showed that the ratio rapidly decreased in ALD patients after treatment. This would suggest the contribution of a direct toxic effect of alcohol on AST/ALT ratio [29].

Total bilirubin, Albumin and Prothrombin time analysed in our study do have utility as components of prognostic indices for patients with advanced ALD. Hyperbilirubinemia in excess of 5mg/dl is one of the findings that connote a poor prognosis as observed in 9 ALD patients in our study. Higher the serum bilirubin in viral hepatitis, the greater is the histologic evidence of hepatocellular damage and longer the course of disease [31].

In ALD, the Maddrey discriminate function, a disease specific prognostic score, has been used to stratify a patient's severity of illness. A score of ≥32 is indicative of severe disease with a 1 month mortality of 30-50% [16] and they are the ones who benefit from corticosteroid therapy [5]. In our study we found the MDF score≥32 among 9 patients with ALD.

CONCLUSION

Our study shows that 6-8 times elevations in GGT and AST/ALT ratio of ≥1.5 together can be used as diagnostic indicators for alcohol induced liver damage. Bilirubin and MDF score have their utility as prognostic indicators as well as to select patients for appropriate mode of therapy.

ABBREVIATIONS

ALD – Alcoholic liver disease
ALT – Alanine aminotransferase
AST – Aspartate aminotransferase
AVH – Acute viral hepatitis
GGT – Gamma glutamyltrasferase
MDF – Maddrey discriminant function
NALD – Non alcoholic liver disease
PT – Prothrombin time

REFERENCES

[1] Singh RB, Ghosh S, Niaz MA, Rastogi V, Wander GS (1998). Validation of tobacco and alcohol intake questionnaire in relation to food intakes for the five city study and a proposed classification for Indians. JAPI, 46(7):587-91.

[2] Narawane NM, Bhatia S, Abraham P, Sanghani S, Sawanth SS (1998). Consumption of country liquor and its relation to Alcoholic liver disease in Mumbai. JAPI, 46(6): 510-513.

[3] Sato N, Lindros KO, Baraona E et al (2001). Sex difference in alcohol related organ injury. Alcohol ClinExp Res, 25:40S-5S.

[4] Mandayam S, Jamal MM, Morgan TR (2004). Epidemiology of alcohol liver disease. Semin Liver Dis, 24(3):217-32.

[5] Walsch K, Alexander G (2000). Alcoholic liver disease. Post grad Med J, 76:280-86.

[6] Mendenhall C, Roselle GA, Gartside P et al (1995). Relationship of protein calorie malnutrition to alcoholic liver disease: a re-examination of data from two Veterans Administrative Cooperative Studies. Alcohol ClinExp Res, 19(3):635-41.

[7] Monzoni A, Masutti F, Saccoccio G, Bellentani S, Tiribelli C, Giacca M (2001). Genetic determinants of alcohol induced liver damage. Mol Med, 7(4):255-62.

[8] Matsuda Y, Tsuchishima M, Ueshima Y, Takase S, Takada A (1993). The relationship between the development of alcoholic liver and pancreatic diseases and the induction of gamma glutamyltransferase. Alcohol AlcoholSuppl, 1B:27-33.

[9] Sharpe PC (2001). Biochemical detection and monitoring of alcohol abuse and abstinence. Ann ClinBiochem, 38:652-664.

[10] Szasz G (1969). A kinetic photometric method for serum gamma glutamyltranspeptidase.Clinchem, 15(2):124-136.

[11] Kachmar JF. Enzymes. In: Fundamentals of clinical chemistry, Tietz NW, 1970, W.B saunders company, Philadelphia: 447-448pp.

[12] Wootton IDP. Microanalysis in Medical Biochemistry. 1964, J and A Churchill Ltd, London: 80pp.

[13] Gowenlock AH (ed). Varley's Practical clinical biochemistry, 1988, Heinmann medical books, London: 407pp.

[14] Harold Varley (ed). Practical clinical biochemistry, 1967, Heinmann medical books, London: 244pp

[15] Quick AJ. Hemorrhagic disease and thrombosis, 1966, 2nd edition, Lee and Fibiger, Philadelphia.

[16] O'Shea RS, Dasarathy S, McCullough AJ (2010). Alcoholic liver disease. Am J Gastroenterol, 105:14-32.

[17] Wise BL and Cockayne S. Enzymes In: Clinical chemistry, Bisho ML, Duben-Von Raufen JL, Fody EP (ed), 1985: JB Lippincott company, Philadelphia: 225pp.

[18] Jacobs WLW (1972). Gamma glutamyltranspeptidase in diseases of the liver, cardiovascular system and diabetes mellitus. ClinClimActa, 38:419-434.

[19] Baral N, Pokhrel S, Lamsal M, Yadav BN, Sah SP (2005). Utility of gamma glutamyltranspeptidase and mean corpuscular volume in alcoholic liver disease. Southeast Asian J Trop Med Public Health, 36(4):1007-10.

[20] Pasqualetti P, Festuccia V, MacCarone C, Di Lauro G, Casale R (1995). Diagnostic value of GGT and MCV in chronic hepatitis of alcoholic aetiology. Minerva Med, 86(10):395-402.

[21] Ryback RS, Eckardt MJ, Felsher B, Rawlings RR (1982). Biochemical and haematological correlates of alcoholism and liver disease. JAMA,248:2261-5.

[22] Wilkinson H.J. The principles and practice of Diagnostic enzymology, 1976: Edward Arnold Ltd, London, 85-87pp.

[23] Sharpe PC, McBride R, Archbold GPR (1996). Biochemical markers of alcohol abuse. Q J Med, 89:137-44.

[24] Maltoff DS, Selinger MJ, Kaplan MM (1980). Hepatic transaminase activity in alcoholic liver disease. Gastroenterology, 8(6):1389-92.

[25] Cohen JA and Kaplan MM (1979). The SGOT/SGPT ratio-An indicator of alcoholic liver disease. Dig Dis Sci, 24(11):835-38.

[26] Borghi E, Melocchi F, Schembri S et al (1991). Alcoholic hepatopathy, deficiency, pathology and serum transaminase. Presentation of 2 clinical cases. Recenti-Prog-Med,82(6):331-333.

[27] Diehl AM, Potter J, Boitnott J, Van Duyn MA, Herlong HF, Mezey E (1984). Relationship between pyridoxal-5-phosphate deficiency and aminotransferase levels in alcoholic hepatitis. Gastroenterology, 86:632-36.

[28] Daeppen JB, Smith KS, Smith TL, Schuckit MA (1999). Characteristics of alcohol dependent subjects with very elevated levels of GGT. J Stud Alcohol, 60:589-94

[29] Nyblom H, Berggren U, Balldin J, Olsson R (2004). High AST/ALT ratio may indicate advanced alcoholic liver disease rather than heavy drinking. Alcohol and Alcoholism, 39(4):336-339.

[30] Sorbi D, Boynton J, Lindor KD (1999). The ratio of AST to ALT: Potential value in differentiating non alcoholicsteatohepatitis from alcoholic liver disease. The Am J Gastroenterol, 94(4):1018-22.

[31] Crawford JM, Gollan JL. Bilirubin metabolism and the pathophysiology of jaundice. In: Diseases of the Liver. Schiff L, Schiff ER (Ed) (1993) JB Lippincott company, Philadelphia: 42pp.